

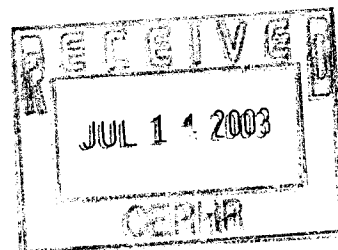
COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR



July 14, 2003

Via E-Mail and Overnight Mail

Dr. Michael Shelby
CERHR
79 TW Alexander Drive
Building 4401
Suite 101-Room 102
Research Triangle Park, NC 27709



Re: Comments on NTP-CERHR Expert Panel Report on Ethylene Glycol

Dear Dr. Shelby:

The American Chemistry Council Ethylene Oxide/Ethylene Glycols Panel (Panel) submits these comments on the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) Expert Panel Report on ethylene glycol (Final Report),¹ the availability of which was announced in the May 15, 2003, *Federal Register*.² The member companies of the Panel comprise the major domestic producers of ethylene glycol in the United States.³

¹ NTP CERHR, NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Ethylene Glycol (May, 2003), available on the Internet at http://cerhr.niehs.nih.gov/news/egpg/EG_Report_Final.pdf.

² 68 Fed. Reg. 26325 (May 15, 2003).

³ The ethylene glycol Panel members of the Ethylene Oxide/Ethylene Glycols Panel are: The Dow Chemical Company, Eastman Chemical Company, Equistar Chemicals, L.P., Huntsman Corporation, and Shell Chemical LP.



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The Panel wishes to commend the NTP CERHR Expert Panel (Expert Panel) and the CERHR staff for their thorough review and summary of the relevant scientific literature. The Panel, however, wishes to bring to the attention of NTP and make a part of the public record two fundamental concerns it has with regard to the discussion in the Overall Conclusions section of the Final Report (Section 5.3). The Panel believes that the Overall Conclusions section of the Final Report should have referred to data presented by the Panel, which shows that estimates of saturation dose in humans and the deicing exposure scenario were based on overly conservative assumptions. Therefore, this section also should have noted that the margin of safety is likely to be even greater than 100 to 1,000. It is particularly important that the Overall Conclusions address these issues given that most readers are likely to focus on this section of the Final Report. The Panel additional comments are set out in the in the attachment to this letter.

The Panel's first fundamental concern is that the discussion in Section 5.3 does not provide a sufficiently clear picture of the conservative nature of the estimate of the ethylene glycol dose, which would result in the buildup of the metabolite, glycolic acid, in humans. For example, as part of the final overall conclusion near the end of the section, Section 5.3 refers to "the dose where saturation of human metabolism is estimated to occur (125 mg/kg bw)." While Section 5.3 near its beginning notes that limited *in vitro* data suggest that saturation of glycolic acid metabolism occurs at approximately 125 mg/kg bw, the discussion as a whole conveys the impression that this is the only driving force for non-linear kinetics for glycolic acid and that this single *in vitro* study accurately reflects a lower saturating dose level for glycolic acid in humans

versus rats. As discussed in the comments on Section 2.1.3.3 in the Attachment, there are other data that indicate that the metabolism of glycolic acid may saturate in humans at a higher concentration than in the rat, not lower. Thus, the Panel believes that Section 5.3 should have referred to and summarized the caveats included in the main body of the Final Report at the top of page 37, as well as the additional caveats and factors discussed by the Panel in its comments on Section 2.1.3.3 in the Attachment, with respect to the 125 mg/kg bw estimate and the suggestion in the Final Report that the human saturation level is 4-fold lower than the ethylene glycol metabolic saturation level in the rat. Accordingly, Section 5.3 should have stated that if 125 mg/kg bw is assumed to be the level of saturation of metabolism in humans, then such an assumption should be qualified by stating that these caveats and factors suggest the level of saturation of metabolism in humans is likely to be higher than 125 mg/kg bw.

Section 5.3 of the Final Report presents an occupational inhalation exposure scenario resulting in a total ethylene glycol exposure burden associated with deicing, presumably of airplanes, of 1.4 mg/kg bw/8 hours. The total exposure burden is based on an assumed ethylene glycol inhalation exposure to 10 mg/m³ for 480 minutes. From the estimated exposure burden, the Final Report concludes that exposures associated with this activity are at least 100- to 1,000-fold lower than those expected to result in metabolic saturation in humans, assuming an estimated human saturation dose of 125 mg/kg bw.

Even assuming an exposure concentration of 10 mg/m^3 and that the human saturation dose is 125 mg/kg bw , the estimated total exposure burden is substantially too high and the actual exposure margins from the saturation dose are substantially greater than the estimated margins of 100- to 1,000 fold. This follows from the fact that the assumption of 8 hours of continuous daily exposure highly overestimates actual exposures. The Panel believes that even under a reasonable worst-case scenario, due to the fact, among other things, that there are substantial gaps in exposure-related activities for a worker between plane deicings, the actual duration of exposure in a day is much less than 8 hours. Based on discussions of Panel representatives with a person who oversees airplane operations, it is estimated that during an 8-hour work shift the actual spraying of the plane with deicing fluid is only between 15 and 20 percent of the work activities for any deicing worker;⁴ however, the actual amount of deicing that occurs during the workday will vary depending upon weather conditions, size of airport, size of the aircraft, and availability of workers. Therefore, 8 hours of continuous exposure to 10 mg/m^3 referenced in the Final Report is not reasonable even for a worst-case scenario.

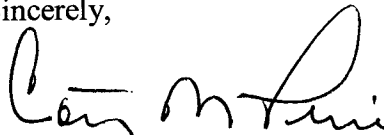
While the Panel understands that these comments will be included as part of the NTP-CERHR monograph on ethylene glycol, the Panel urges that the NTP "brief" section of the monograph reflect the above discussion. The Panel appreciates your consideration of this letter

⁴ Personal communication between Mark Aldrich, General Manager, Integrated Deicing Systems, Westminster, CO and Cheryl Wizda, Senior Product Development Specialist, The Dow Chemical Company, S. Charleston, WV (June 16, 2003).

Dr. Michael Shelby
July 14, 2003
Page 5

and the supplemental comments in the attachment. If you have questions, please call William P. Gullledge, Manager of the Ethylene Oxide/Ethylene Glycols Panel, at (703) 741-5613.

Sincerely,

A handwritten signature in black ink, appearing to read "Courtney M. Price". The signature is fluid and cursive, with the first name "Courtney" written in a larger, more prominent script than the last name "Price".

Courtney M. Price
Vice President, CHEMSTAR

Attachment

ATTACHMENT

ADDITIONAL COMMENTS BY THE AMERICAN CHEMISTRY COUNCIL ETHYLENE OXIDE/ETHYLENE GLYCOLS PANEL

The American Chemistry Council Ethylene Oxide/Ethylene Glycols Panel (Panel) submits these additional comments on the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) Expert Panel Report on the Developmental and Reproductive Toxicity of Ethylene Glycol (Final Report). These comments are organized according to the section or subsection number in the Final Report.

As a preliminary matter, the Panel commends the NTP CERHR Expert Panel for its thorough review of ethylene glycol and its summary of the relevant scientific literature. This attachment addresses several factual and related issues that the Panel wishes to be included as part of the NTP-CERHR monograph on ethylene glycol.

1.2.3 Occurrence. The Panel recommends that the following corrections and clarifications to this section be made:

- The statement that in 2000, the Toxics Release Inventory (TRI) reported an estimated 7.1 million pounds of ethylene glycol released to the atmosphere from U.S. manufacturing and processing facilities is inconsistent with the actual numbers in the 2000 TRI database. The 2000 database states that (rounding up) the *total release* into the *atmosphere*

was 4.7 million pounds, and the total on-site releases totaled 7.1 million pounds. The TRI database indicates that in 2001 the total release into the atmosphere was 4.9 million pounds and the total on-site releases were 7.6 million pounds. These figures should also be included in Section 1.4 (page 10), which is ambiguous.

- The first sentence on page 3, second paragraph, should state that Health Canada cited a “study” instead of “studies,” to reflect accurately the sentence that follows, which acknowledges that the information comes from a single 1994 study.

- Additionally, the Panel wishes to note that the Sciences International data on the Alberta facility in Table 1-2, page 5, represent an extreme plausible worst-case scenario. The maximum 24-hour average concentration of 134 µG/MG was determined to be 134 µG/MG at both the maximum nearby residence and at the company outer border, thus combining to create the most extreme plausible worst case of intermittent events.

1.2.4 Human Exposure.

1.2.4.1 General Population Exposure. Page 6, paragraphs 2 and 3. The discussion in the Final Report states that it is not known if ethylene oxide is used to sterilize foods sold in the United States. To the Panel’s knowledge, the only major food use for

sterilization with ethylene oxide in the United States is spices, including herbs and other seasonings.

Page 6, last paragraph: A significant part of the exposure estimate as a worst case scenario for persons living next to an industrial point source consisted of ingestion of ethylene glycol in soil. The estimated exposure by this route was based on a value of 4,290 mg/kg.¹ The Panel believes that the Final Report should not have used this value in its exposure assessment because the soil concentration was based on a personal reference (personal communication AEP, 1996). The Panel contacted the personal reference who indicated he had no data to verify the stated concentration. Moreover, the Panel cannot understand how under normal industrial situations a value of 4,290 mg/kg in soil near an industrial point of discharge is feasible. If ethylene glycol deposition is calculated in an ISCST3 screening study, and it is assumed that all emissions condense and form 10 micron diameter particles/droplets, the maximum predicted annual deposition flux is 5.2 g/m² per year at 1.8 kilometers from the source. The 24-hour average deposition would be 14 times lower. If one assumes that annual deposition is spread uniformly in the top 2.5 centimeters of soil (average density of 1.3 g/cm³), then the contaminant concentration is 0.15 mg/kg of soil. This is only 0.004% of the maximum concentration of 4,290 mg/kg in soil near an industrial point source. Therefore, the Final Report should have estimated that a worst-case total exposure scenario (inhalation and soil), with the very low soil concentration, would be only 20-60 µg/kg bw/day.

¹ See Reference (19), at 116 (Table 12, ft. 8).

1.2.4.2 Occupational Exposure. Page 9. This section of the Final Report (occupational exposure section) should state that ethylene glycol has a low vapor pressure (*i.e.*, 0.06 m Hg at 20°C).

2.1.3 Metabolism.

2.1.3.1 Humans. Page 20, second full paragraph (Strengths/Weaknesses).

The data used to estimate human burden by ethylene glycol and glycolic acid are not appropriate for calculating ethylene glycol and glycolic acid burdens at the much lower exposure concentrations occurring at workplaces because all these data had been collected in poisoned patients who had undergone medical treatment, which alters the disposition of ethylene glycol metabolites. Furthermore, workplace exposures, unlike the poisoning cases, result in effective doses that are below levels leading to saturation of clearance processes.

Page 20, middle of last paragraph, carry-over to top of page 21 (“Further, there is only” through end of this paragraph). The criticism of “sketchy information on the amount of ethylene glycol dosed and available for inhalation” concerning the submitted manuscript “Carstens *et al.* (37)” is unwarranted. As Dr. J. Filser discussed in his oral comments at the NTP-CERHR Expert Panel meeting on February 11-13, 2003, the criticism on the methodology was not justified. The appended revised and accepted paper² contains all of the required information, proving that practically all of the ethylene glycol dosed into the inhalation vessel was inhaled. The criticism of the application procedure (16 injections into the vessel over 4 hours every 15 minutes for

inhalation) is very similar to a continuous steady exposure considering the 8-fold longer half-life of ethylene glycol (about 2 hours) and therefore such criticism is also unwarranted. This is also mentioned in the accepted manuscript. The Panel also provides the following additional comments on this paragraph:

- In response to the Final Report's criticism regarding the detection limit, the limit of detection for "unchanged ethylene glycol exhalation" is obtained by multiplying the detection limit given in the accepted manuscript for water ($0.3 \mu\text{mol/L} = 0.3 \text{ nmol/ml}$) with the amount of water (5 ml) injected into the gas bag (*see* Materials and Methods of appended manuscript). The result gives the detection limit in question (1.5 nmol). This amount is about 10^{-6} times smaller than the amount inhaled.
- The criticism of not having collected exhaled $^{13}\text{CO}_2$ is unfounded. As shown in Fig. 1 of Marshall and Cheng (1983), it was necessary to collect the exhaled CO_2 for up to 6 days to quantify the share of ethylene glycol being converted to CO_2 in a small animal (the rat). In the much larger human body, an approximately 40-fold longer collection period would be required. In the Panel's view it would be unacceptable to subject a person to the experimental conditions of the study and to collect continuously the exhaled air for many days and nights. Further, all of these efforts would

² Carstens, J., G.A. Csanády, T.H. Faller, J.G. Filser. "Human inhalation exposure to

only demonstrate the well-known fact that ethylene glycol metabolites enter the intermediary metabolism, which finally leads to the production of CO₂.

Page 21, second paragraph (Utility, (Adequacy), etc). The following statement is erroneous: “The urinary output data shown in Table 2-3 suggests that only about 10% of ethylene glycol was converted to glycolic acid.” This follows from the fact that a share of the produced glycolic acid is immediately further metabolized before becoming bioavailable. A more appropriate statement would be that only about 10% of ethylene glycol was bioavailable as glycolic acid. Further, it should be emphasized that the “relatively low and spread-out inhalation exposures” correspond to workplace exposures in contrast to the uptake of high doses (*e.g.* suicidal).

Page 22, Table 2-3. There are certain errors that should be corrected in this table.

- The age of Subject B is 44 years, not 55 years.
- Units of “Estimated total dose” are incorrect. The units of the amount taken up are mmol and mg/kg.

2.1.3.2 Animals. Page 28, “Utility.” Based on the comment that “The utility of the Pottenger, *et al.* (44) study for assessing human fetal exposure is uncertain,” it

ethylene glycol.” *Arch. Toxicol.* (accepted Mar. 12, 2003) (appended to this Attachment).

appears that the objective of the study and its value were misunderstood. The goal of the study was not to assess human fetal exposure, but rather to better characterize maternal and embryonic dosimetry during a stage of development *in the rat* known to be highly sensitive to ethylene glycol.

Therefore, the Panel believes that the second sentence of this paragraph should have been revised to read (changes in italics): “*Although* the study was limited to a narrow window of gestation, gd 10-11, *it is* a sensitive time frame for ethylene glycol developmental toxicity in the Sprague-Dawley rat and *exposure at this time induces most of the developmental effects inducted by gd 6-15 exposure (122).*”

Page 31, bottom paragraph in bold. The Panel believes that the discussion regarding the higher concentrations of ethylene glycol and, in particular, glycolic acid, in the embryos relative to maternal blood, would have been more complete if the following language, indicated by italics, had been added to the text: “*While not completely understood, the trapping of weak acids in rat and mouse embryos is a well established phenomenon seen with a variety of weak acid compounds.* It may also be possible that metabolism of ethylene glycol to glycolic acid occurs in the embryo and thus contributes to the greater internal dose of glycolic acid in the conceptus. *However, studies with isolated rat conceptuses exposed in vitro to ethylene glycol (123) found no evidence of in situ metabolic conversion.*”

2.1.3.3 In Vitro Metabolism Studies. On pages 35-37 of the Final Report, the *in vitro* comparative metabolism study of Bartels (2001) is discussed for its utility in the

evaluation of risks to human reproduction. The Final Report, however, makes an inference from this study that the ethylene glycol dose in humans resulting in saturation of glycolic acid metabolism may be 4-fold lower than the dose resulting in glycolic saturation in rats -- *i.e.*, 125 mg/kg bw for humans versus an ethylene glycol bolus dose of 500 mg/kg bw for rats. The Final Report (at top of page 37) provided several caveats about this extrapolation, however, the extrapolation was, nevertheless, utilized in the report's final, Overall Conclusions (Section 5.3) where this extrapolated metabolic saturation dose level (125 mg/kg) was compared to estimated exposure levels. While the results of this comparison indicated a significant margin of safety for the developmental toxicity of ethylene glycol, there are additional considerations that were not addressed by CERHR in their *in vitro* to *in vivo* extrapolations that should have been included in the Final Report that would affect the inference that glycolic acid could build up in humans at dose levels as low as 125 mg/kg bw.

First, the metabolism of glycolic acid is but one driving force in the overall disposition of glycolic acid. Glycolic acid concentrations in blood and tissues are affected by a balance of processes leading to the formation of glycolic acid from ethylene glycol and those that are involved in its clearance, which includes elimination in urine as well as metabolism. These factors would make potentially significant contributions to glycolic acid levels in blood and tissues and affect this inference of a "saturable dose" of 125 mg/kg.

Furthermore, the differences between rat and human apparent K_m 's for glycolic acid metabolism, which the Final Report used to extrapolate saturating dose levels, may not be as significant as implied in the single study of Bartels (2001). Additional studies by Booth and

Watson (1999b) on the metabolism of glycolic acid by female rat and human liver slices under dynamic culture conditions reported that the K_m was 0.72 mM for humans which is 2.4-fold higher than the K_m in rats (0.3 mM), just the opposite of the findings of Bartels (2001). Given that two different studies yielded opposite results, it remains difficult to assess whether or not there really are significant differences in the overall rates of metabolism *in vitro*, much less *in vivo*. Based on these considerations, the Final Report also should have stated that there are data that indicate that the metabolism of glycolic acid may saturate in humans at a higher concentration in the rat, not lower. This statement also should have been included in other parts of the Final Report, including the Summary and Overall Conclusions section (Section 5.3).

2.1.3.4 Developmental- and Species-Specific Variations in Metabolism and Enzyme Activities.

2.1.3.4.4 Inter-Individual Variability Due to Genetic Polymorphisms and Hereditary Metabolic Disorders. When the Final Report discusses in this section the potential importance of various isoforms or polymorphisms of alcohol and aldehyde dehydrogenases as they relate to the toxicity of ethanol, the Final Report makes the scientifically unsound assumption that the same degree of importance can be extended to ethylene glycol, which clearly demonstrates a different affinity for the enzymes involved in its metabolism.

2.6 Summary.

2.6.1.2 Exposure Route and Dose Rate Effects on Metabolic Saturation.

For the reasons explained in the comments on Section 2.1.3.3 above, the Final Report also should have stated in this section that there are data that indicate that the metabolism of glycolic acid may saturate in humans at a higher concentration than in the rat, not lower.

5.0 Summaries, Conclusion, and Critical Data Needs.

5.1 Summary and Conclusions of Reproductive and Developmental

Hazards.

5.1.1 Developmental Toxicity. Page 121. For the reasons

discussed in the cover comment letter and above in the comments on Section 2.1.3.3, the Final Report should also have stated in this section that there are data that indicate that the metabolism of glycolic acid may saturate in humans at a higher concentration than in the rat, not lower.

Page 120. In the discussion regarding metabolic saturation, the Panel believes rats and mice should have been mentioned separately, as these species show pharmacokinetic differences that correspond to their developmental toxicity profile. This could have been accomplished by the minor edits to the following sentence (added text in italics): “This

saturation occurs beginning at *oral bolus* doses as low as 150 mg/kg in *mice* and ≥ 500 mg/kg in *rats*.”

5.3 Overall Conclusions. As discussed in the cover letter, there are certain caveats and other considerations in the body of the Final Report (top of page 37) and additional caveats and factors discussed in the above comments on Section 2.1.3.3 concerning the Final Report’s estimate that saturation of glycolic acid metabolism occurs at approximately 125 mg/kg bw in humans and the suggestion that the human saturation level is 4-fold lower than the ethylene glycol metabolic saturation level in the rat. The Panel believes that this section of the Final Report should have referred to and summarized those caveats and factors. In addition, the Panel believes that this section, considering those caveats and factors, also should have stated that there are data that indicate that the metabolism of glycolic acid may saturate in humans at a higher concentration than in the rat, not lower. Accordingly, this section of the Final Report should also have explained that the margins of exposure from metabolic saturation indicated on page 123, while large, could be somewhat larger.

The Panel believes that this section, for purposes of clarity, also should have made the broader conclusion that existing data on human exposure levels indicate that developmental toxicity is of negligible concern.

The Panel also notes that the two calculated values of 0.8 mg/kg within 15 minutes and 1.4 mg/kg within 8 hours do not represent the estimated “burden,” as stated, but only the estimated amounts inhaled. These values could represent worst-case estimates of the

body burden only by ignoring the fact that ethylene glycol is normally being metabolized and the overestimate of exposure discussed below.

For the reasons described in greater detail in the comment letter, the Panel believes that, even assuming an exposure concentration of 10 mg/m^3 , the assumption of 8 hours of continuous daily exposure for deicing workers highly overestimates actual exposures and is not reasonable for a worst-case scenario. Accordingly, even assuming an exposure concentration of 10 mg/m^3 and that the human saturation dose is 125 mg/kg bw , the actual exposure margins from the saturation dose are substantially higher than the estimated margins of 100- to 1,000-fold based on these exposure considerations alone. The Panel is collecting more information on this type of exposure.

Attachment

Toxicokinetics and Metabolism

Human inhalation exposure to ethylene glycol

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Abstract Two male volunteers (A and B) inhaled 1.43 and 1.34 mmol, respectively, of vaporous ^{13}C -labeled ethylene glycol ($^{13}\text{C}_2\text{-EG}$) over 4 h. In plasma, $^{13}\text{C}_2\text{-EG}$ and its metabolite $^{13}\text{C}_2\text{-glycolic acid}$ ($^{13}\text{C}_2\text{-GA}$) were determined together with the natural burden from background GA using a gas chromatograph equipped with a mass selective detector. Maximum plasma concentrations of $^{13}\text{C}_2\text{-EG}$ were 11.0 and 15.8 $\mu\text{mol/l}$, and of $^{13}\text{C}_2\text{-GA}$ were 0.9 and 1.8 $\mu\text{mol/l}$, for volunteers A and B, respectively. Corresponding plasma half-lives were 2.1 and 2.6 h for $^{13}\text{C}_2\text{-EG}$, and 2.9 and 2.6 h for $^{13}\text{C}_2\text{-GA}$. Background GA concentrations were 25.8 and 28.3 $\mu\text{mol/l}$ plasma. Unlabeled background EG, GA and oxalic acid (OA) were detected in urine in which the corresponding ^{13}C -labeled compounds were also quantified. Within 28 h after the start of the exposures, 6.4% and 9.3% $^{13}\text{C}_2\text{-EG}$, 0.70% and 0.92% $^{13}\text{C}_2\text{-GA}$, as well as 0.08% and 0.28% $^{13}\text{C}_2\text{-OA}$ of the inhaled amounts of $^{13}\text{C}_2\text{-EG}$, were excreted in urine by volunteers A and B, respectively. The amounts of $^{13}\text{C}_2\text{-GA}$ represented 3.7% and 14.2% of background urinary GA excreted over 24 h (274 and 88 μmol). The amounts of $^{13}\text{C}_2\text{-OA}$ were 0.5% and 2.1% of background urinary OA excreted over 24 h (215 and 177 μmol). From the findings obtained in plasma and urine and from a toxicokinetic analysis of these data, it is highly unlikely that workplace EG exposure according to the

German exposure limit (MAK-value 10 ppm EG, 8 h) could lead to adverse effects from the metabolically formed GA and OA.

Keywords Ethylene glycol · Glycolic acid · Oxalic acid · Human · Risk · Metabolism · Toxicokinetics

This publication is dedicated to Prof. Dr. Dr. Hermann M. Bolt on the occasion of his 60th birthday.

Introduction

Ethylene glycol (EG) is a widely used liquid with a low vapor pressure of 0.08 mbar at 20°C (DFG 1991). It is employed in antifreeze formulations, de-icing of airplanes, production of polyesters, and a series of other applications.

High doses of EG, a simplified metabolic scheme of which is given in Fig. 1, show various toxic effects. Symptoms related to the central nervous system, metabolic acidosis, nephrotoxic and embryotoxic properties are characteristic (Andrews and Snyder 1991). Nephrotoxicity is ascribed to acidic metabolites as glycolic acid (GA) and oxalic acid (OA) (reviewed, for example, in LaKind et al. 1999). Both acids also showed developmental toxicity in rat embryo cultures (Klug et al. 2001). In vivo, EG-exposed rats produced only minor amounts of OA in comparison with those of GA (Frantz et al. 1996). Embryotoxicity in EG- and GA-treated rats was related to GA (Carney et al. 1999). In rats, receiving EG in their food over 2 years, no-observed-effect-levels (NOELs) for EG-induced nephrotoxicity were 0.2% in food [about 80 mg/kg body weight (bw) per day for 2 years; Blood 1965] and 0.5% (about 200 mg/kg bw per day for 2 years; DePass et al. 1986). For B6C3F1 mice, the corresponding NOEL was 0.6% in food (about 1500 mg/kg bw per day for 2 years) (National Toxicology Program 1993, cited in LaKind et al. 1999). NOELs for developmental toxicity have been reported to be 500 mg EG/kg bw per day in CD rats and 150 mg/kg bw per day in CD-1 mice (administration by gavage from gestation day 6 to gestation day 15; both species see Neeper-Bradley et al. 1995). For rats, the NOEL dose of 80 mg/kg bw can be calculated to lead to a maximum GA concentration in blood of 144 $\mu\text{mol/l}$ (linear extrapolation from the maximum GA blood concentration of 271 $\mu\text{mol/l}$ reached after a dose of 150 mg EG/kg bw; Pottenger et al. 2001).

The dose of 500 mg EG/kg bw resulted in a maximum GA concentration of 1723 $\mu\text{mol/l}$ blood (Pottenger et al. 2001).

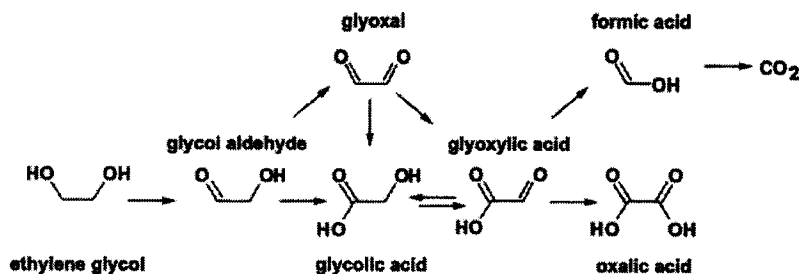


Fig. 1. Simplified scheme of ethylene glycol metabolism

In the US National Occupational Exposure Survey, conducted during 1981–1983, it was estimated that about 1.5 million workers were potentially exposed to EG in the USA, each year (National Institute of Occupational Safety and Health 1990, cited in IPCS 2002). Exposure may lead to uptake of EG. Only limited experimental or occupational data are available on humans exposed to EG in spite of its occupational relevance. Urinary EG was found in volunteers exposed (20–22 h/day, 30 days) to weekly air concentrations of EG between 17 and 49 mg/m^3 (Wills et al. 1974) in workers using EG for de-icing of airplanes (Gérin et al. 1997), and in motor servicing workers (Laitinen et al. 1995). None of these studies established a reliable correlation between EG concentrations in urine and air. In order to obtain such a relationship and to enable a comparison between background and additional exposure-derived burdens, we determined EG, GA and OA levels and those of their $^{13}\text{C}_2$ -labeled analogues in plasma and urine of human volunteers inhaling small amounts of vaporous $^{13}\text{C}_2$ -EG at exposure conditions below the German MAK-value (maximum workplace concentration of EG 10 ppm = 25.7 mg/m^3 ; DFG 1991).

Materials and methods

Chemicals

$^{13}\text{C}_2$ -EG (99 atom% ^{13}C) was obtained from Aldrich (Steinheim, Germany). All other chemicals were of analytical grade.

Exposure experiments

The study was approved by the ethics committee of the Technical University, Munich.

Two healthy, non-smoking, male volunteers (volunteer A: 54 years old, 96 kg; B: 44 years old, 57 kg) avoided oxalate- and ascorbate-rich foods for 2 days before the individual experiments. On the exposure days, each volunteer inhaled vaporized $^{13}\text{C}_2\text{-EG}$ from an all-glass vessel (250 ml, Fig. 2). Vapors of $^{13}\text{C}_2\text{-EG}$ were generated by repeatedly (16 times at intervals of 15 min over 4 h) injecting small amounts of liquid $^{13}\text{C}_2\text{-EG}$ (6.6 μl each) via a septum into the closed vessel using a calibrated 10 μl syringe (SGE, Darmstadt, Germany). The vessel was warmed to about 140°C (boiling point of EG 198°C) to facilitate the evaporation. At the beginning of the time intervals, the volunteer inhaled the generated vapor via the mouth. To start the inhalation, the volunteer successively opened the outlet and then the inlet valve and took a deep breath. The inhaled air stream entered the vessel by the inlet valve and transported the $^{13}\text{C}_2\text{-EG}$ vapor into the respiratory tract. Immediately thereafter, the valves were closed. This inhalation procedure was repeated three to four times. At selected time points exhaled air was collected in gas bags (polyethylene-coated aluminum bags; Linde, Unterschleißheim, Germany) in order to quantify the fraction of exhaled $^{13}\text{C}_2\text{-EG}$. The gas bags were sealed immediately after collection. At the end of the exposure period (after 4 h) residual $^{13}\text{C}_2\text{-EG}$ was determined from the vessel. For this purpose, 10 ml deionized water was injected into the vessel via the septum. The vessel was thoroughly shaken in order to wet all glass surfaces. Correspondingly, to determine the amounts of exhaled $^{13}\text{C}_2\text{-EG}$, 5 ml deionized water was injected into the gas bags. The aqueous solutions were stored at -80°C until analyzed for $^{13}\text{C}_2\text{-EG}$.

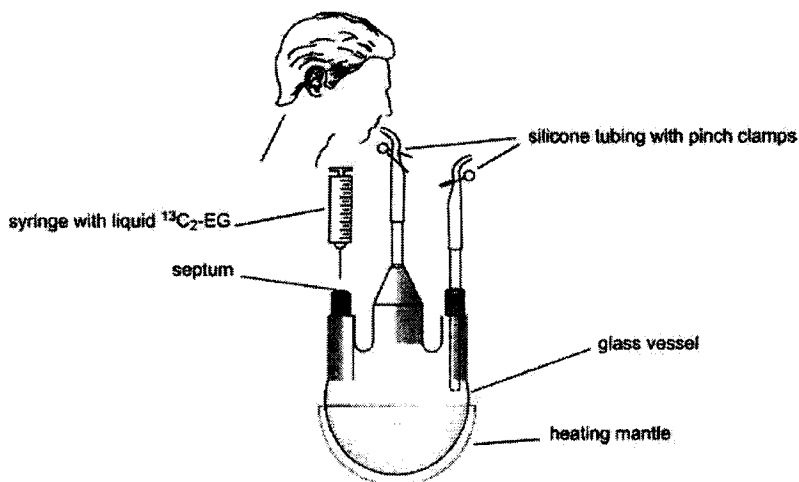


Fig. 2. Schematic set-up for inhalation exposure to ethylene glycol ($^{13}\text{C}_2\text{-EG}$) vapor

Prior to the exposure, an intravenous indwelling cannula (B. Braun, Melsungen, Germany) was positioned in the cubital vein. Samples of about 5 ml venous blood were collected before the start of the exposure, during the exposure at each time intervals of 15 min (about 7 min following each inhalation process), and up to 4 h post-exposure. Blood pH was determined in each sample. Plasma, obtained by centrifugation, was stored at -80°C until analyzed for labeled and unlabeled EG and GA. All urinary fractions were collected immediately prior to start of exposure and up to 30 h thereafter. Urinary pH was monitored immediately in each fraction, which was subsequently acidified. Then, fractions were stored at 4°C for up to 24 h. Within this period, specimens were taken for the determination of labeled and unlabeled OA. Immediately thereafter, fractions were stored at -80°C until analyzed for labeled and unlabeled EG and GA. A 5-week stability test demonstrated that the storage duration did not influence the extent of recovery of the analytes.

Analysis of ethylene glycol, glycolic acid and oxalic acid by gas chromatography

A detailed description of the analytical procedures is in preparation and will be submitted elsewhere. Therefore, only a brief description is given.

A gas chromatograph equipped with a mass selective detector (GC/MSD) was used for all analyses (GC: HP5890 Series II with cool-on-column inlet equipped with a HP-5MS column, 30 m length, 0.25 mm i.d., 0.25 mm film, carrier gas Helium; MSD: HP5972, electron impact ionization, 70 eV, selective ion monitoring). The complete system was obtained from Agilent Technologies, Waldbronn, Germany.

Sample preparation for ethylene glycol analysis

Both, propylene glycol and 1,3-propanediol served as internal standards in parallel. EG and the standards in the samples were derivatized with *n*-butylboronic acid, extracted with ethyl acetate and analyzed (limits of quantification in plasma: EG $7.6\text{ }\mu\text{mol/l}$, $^{13}\text{C}_2\text{-EG}$ $0.6\text{ }\mu\text{mol/l}$; in urine: EG $1.1\text{ }\mu\text{mol/l}$, $^{13}\text{C}_2\text{-EG}$ $0.1\text{ }\mu\text{mol/l}$; in water $^{13}\text{C}_2\text{-EG}$ $0.3\text{ }\mu\text{mol/l}$).

Sample preparation for glycolic acid analysis

Deuterated succinic acid ($\text{D}_6\text{-SA}$) and 2-hydroxyisovaleric acid were added as internal standards to plasma and to urine, respectively. The corresponding samples were deproteinized with acetonitrile (only plasma) and dried using a vacuum concentrator. The residues were

treated with *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA), and the derivatives were analyzed (limits of quantification in plasma: GA 1.2 $\mu\text{mol/l}$, $^{13}\text{C}_2$ -GA 0.8 $\mu\text{mol/l}$; in urine: GA 32.6 $\mu\text{mol/l}$, $^{13}\text{C}_2$ -GA 2.9 $\mu\text{mol/l}$).

Sample preparation for oxalic acid analysis

Succinic acid and D₆-SA served as internal standards for the determination of OA and of $^{13}\text{C}_2$ -OA, respectively. After adding these standards, the samples were acidified and extracted with ethyl acetate. The residues obtained after drying with a vacuum concentrator were silylated with MTBSTFA and the derivatives were analyzed (limits of quantification in urine: OA 17.2 $\mu\text{mol/l}$, $^{13}\text{C}_2$ -OA 0.2 $\mu\text{mol/l}$).

Results

The volunteers did not report any effects related to the exposure. The inhaled $^{13}\text{C}_2$ -EG was completely taken up from the inhaled air as proven by its absence in the expired air. Therefore, the amount taken up during the entire exposure period equaled the difference between the sum of the administered doses and the residual $^{13}\text{C}_2$ -EG, which was determined in the glass vessel at the end of exposure. The pH measurements in blood and urine showed normal physiological values being 7.45 ± 0.024 (volunteer A) and 7.39 ± 0.027 (B), and ranging from 6 to 7.5 (A) and from 6 to 8 (B). In plasma of the volunteers, $^{13}\text{C}_2$ -EG, $^{13}\text{C}_2$ -GA and GA could be determined. A background concentration of EG was not found at a limit of quantification of 7.6 $\mu\text{mol/l}$. This high value probably resulted from interference by other compounds in plasma. There was no interference for the determination of $^{13}\text{C}_2$ -EG. In urine of both volunteers, labeled and unlabeled EG, GA and OA were quantifiable.

Table 1 shows the inhaled doses of $^{13}\text{C}_2$ -EG, both total (in millimoles) and normalized for body weight of each volunteer (as milligrams per kilogram body weight).

[Table 1. will appear here. See end of document.]

Figure 3 depicts the concentration–time courses of $^{13}\text{C}_2$ -EG in plasma during and after exposure. The ratio of the maximum $^{13}\text{C}_2$ -EG concentration found in volunteer A to that in B reflects that of the doses, as given in Table 1. The half-lives of $^{13}\text{C}_2$ -EG, derived from the elimination phase of Fig. 3, are 2.1 h (A) and 2.6 h (B). Twenty-four hours after start of the exposure $^{13}\text{C}_2$ -EG was no longer detectable. With $^{13}\text{C}_2$ -EG half-lives of ≥ 2 h and dosing intervals of 15 min, the exposure can be considered as quasi-continuous.

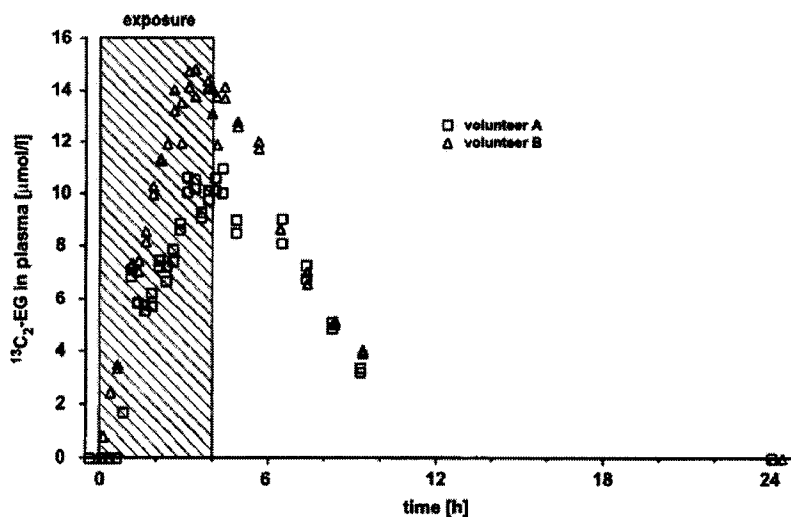


Fig. 3. Plasma concentrations of $^{13}\text{C}_2$ -ethylene glycol ($^{13}\text{C}_2$ -EG) during and after a 4-h inhalation exposure to $^{13}\text{C}_2$ -EG vapor

Figure 4 displays the concentration-time courses of $^{13}\text{C}_2$ -GA in plasma during and after exposure. The maximum $^{13}\text{C}_2$ -GA concentrations were reached shortly after the end of exposure (for volunteer A about 1 h; for B 0.5 h). These short time-spans are in agreement with the observation that the $^{13}\text{C}_2$ -GA half-lives of 2.9 h (A) and 2.6 h (B) were similar (A) or identical (B) with those of $^{13}\text{C}_2$ -EG. The maximum $^{13}\text{C}_2$ -GA concentration was lower in volunteer A than in B, as was also observed for the maximum $^{13}\text{C}_2$ -EG concentration (see above). The background plasma concentrations of GA were 25.8 ± 3.7 and $28.3 \pm 2.8 \mu\text{mol/l}$ for volunteer A and B, respectively.

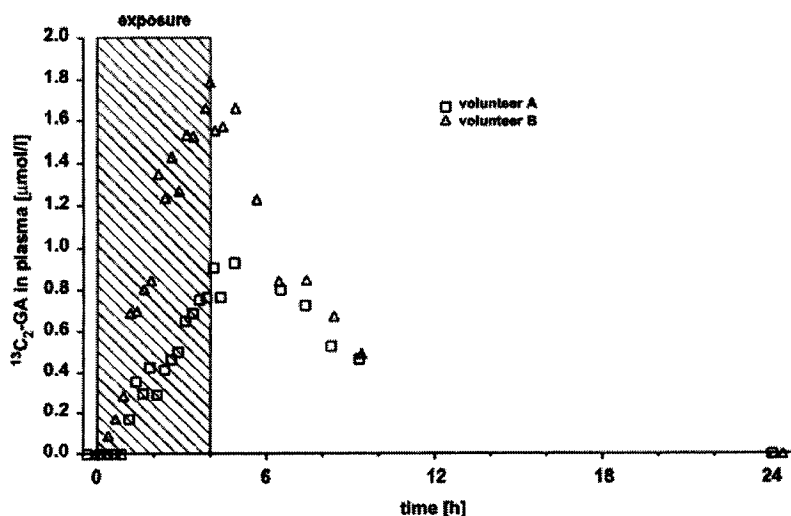


Fig. 4. Plasma concentrations of $^{13}\text{C}_2$ -glycolic acid ($^{13}\text{C}_2$ -GA) in plasma during and after a 4-h inhalation exposure to $^{13}\text{C}_2$ -ethylene glycol vapor

Figures 5, 6 and 7 depict the cumulative amounts of unlabeled (background) and labeled EG, GA and OA excreted in the urine that was collected during and after exposure.

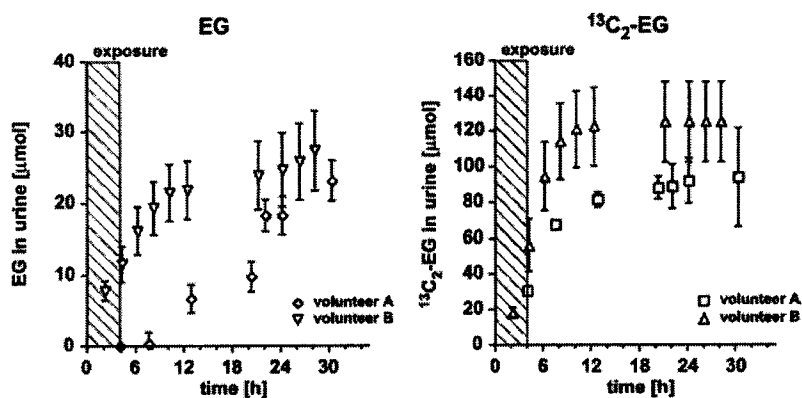


Fig. 5. Background ethylene glycol (EG) and $^{13}\text{C}_2$ -EG in urine during and after a 4-h inhalation exposure to $^{13}\text{C}_2$ -EG vapor, expressed as cumulative excretion (means \pm SD, $n=3$)

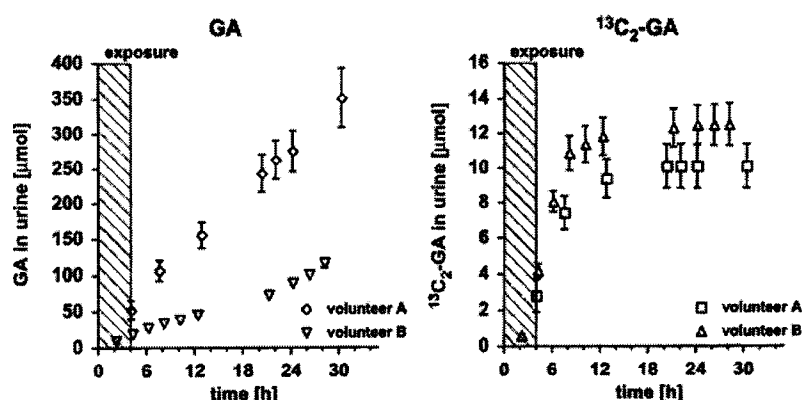


Fig. 6. Background glycolic acid (GA) and $^{13}\text{C}_2$ -GA in urine during and after a 4-h inhalation exposure to $^{13}\text{C}_2$ -ethylene glycol vapor, expressed as cumulative excretion (means \pm SD, $n=3$)

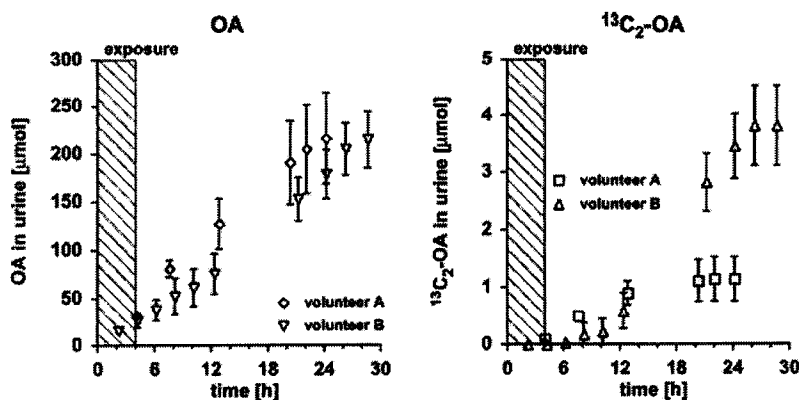


Fig. 7. Background oxalic acid (OA) and $^{13}\text{C}_2\text{-OA}$ in urine during and after a 4-h inhalation exposure to $^{13}\text{C}_2$ -ethylene glycol vapor, expressed as cumulative excretion (means \pm SD, $n=3$)

Table 2 shows the amounts of $^{13}\text{C}_2\text{-EG}$, $^{13}\text{C}_2\text{-GA}$ and $^{13}\text{C}_2\text{-OA}$ in urine, expressed as a percentage of the inhaled dose of $^{13}\text{C}_2\text{-EG}$ (see Table 1). Obviously, EG is predominantly biotransformed to other metabolites presumably via intermediary metabolism. This conclusion is supported by data from rat studies. Following a 30-min inhalation exposure of Fischer-344 rats to gaseous $^{14}\text{C-EG}$ at a mean air concentration of 12.5 ppm, Marshall and Cheng (1983) determined the amounts of exhaled $^{14}\text{CO}_2$. From the initial ^{14}C -burden, about 30% was exhaled as $^{14}\text{CO}_2$ within the first 12 h. This metabolite amounted to 60% after 4 days.

[Table 2. will appear here. See end of document.]

Table 3 gives the amounts of background GA and OA excreted over 24 h. It shows also the relative increases in these acids as labeled metabolites formed from inhaled $^{13}\text{C}_2\text{-EG}$, the dose of which was 1.43 mmol (A) and 1.34 mmol (B).

[Table 3. will appear here. See end of document.]

Discussion

Background values

Ethylene glycol

Background EG was quantifiable in urine (volunteer A: $18.2 \mu\text{mol}/24 \text{ h}$; B: $24.5 \mu\text{mol}/24 \text{ h}$). These urinary EG excretions can be compared with ratios of urinary EG to urinary creatinine reported by other groups for non-exposed persons (Laitinen et al. 1995, 1997; G  rin et al 1997; Letzel et al. 2000). Using these ratios and considering a normal creatinine excretion

of 1.8 g/24 h in healthy men (Geigy Documenta 1975), EG excretions can be calculated to be between 0 and 77 $\mu\text{mol}/24\text{ h}$. These amounts match with those measured in volunteers A and B. In the study of Wills et al. (1974) EG background concentrations have been reported for both serum (1500–3400 $\mu\text{mol}/\text{l}$) and urine (260–1200 $\mu\text{mol}/\text{l}$). The values seem to be very high for unexposed controls, in the light of the other published data on urinary EG. The difference might result from the rather unspecific methodology used by Wills et al. (1974), which was based on the periodate oxidation of EG to formaldehyde used by Russell et al. (1969). Since treatment with this reagent transforms a number of substances present in biological material into formaldehyde (Russell et al. 1969) an overestimation of EG probably occurred. Background EG could result from several sources. Endogenous and environmental ethylene and ethylene oxide could contribute via metabolism to background EG (reviewed in IARC 1994; Filser et al. 1994). A relevant EG source could be ethylene and ethylene oxide uptake via smoking (e.g. Törnqvist et al. 1986; Filser et al. 1992). A series of further possible sources for background EG, including food and consumer products, are discussed in IPCS (2002).

Glycolic acid

The GA background concentrations in plasma determined in the present study are in agreement with those (6.6–32.9 $\mu\text{mol}/\text{l}$) published by Chalmers et al. (1984). Other authors have reported lower GA background concentrations (between 4.4 and 12.2 $\mu\text{mol}/\text{l}$) in plasma of fasted subjects (Maeda-Nakai and Ichiyama 2000), and in plasma-ultrafiltrate of fasted (Hagen et al. 1993) and non-fasted individuals (Petrarulo et al. 1991). Urinary background amounts of GA, excreted over 24 h (Table 3) are consistent with values from 75 to 1220 $\mu\text{mol}/24\text{ h}$ for healthy humans, published by a series of other groups (review by Petrarulo et al. 1998; Niederwieser et al. 1978; Marangella et al. 1992; Holmes et al. 1993; Maeda-Nakai and Ichiyama 2000). Only one publication reported a wider range from 0 to 1400 $\mu\text{mol}/24\text{ h}$ (Hagen et al. 1993). Background GA can originate from several sources. GA is formed in the catabolism of proteins (Holmes et al. 1993) and carbohydrates (McWinney et al. 1987). It can also be taken up directly via food since it has been detected as a natural constituent of vegetables, fruits and meat (Harris and Richardson 1980). These authors estimated a daily total GA intake via food of 33 mg for an adult. Furthermore, GA is an ingredient of certain cosmetic products (reviewed in NICNAS 2000).

Oxalic acid

Unfortunately, we were not able to quantify OA in plasma because the recovery in plasma was not reproducible. The reason for this behavior is unclear. Recently, several methods for the determination of the OA background concentrations in plasma have been summarized (reviewed in Petrarulo et al. 1998; further citations in Hönow et al. 2002). The reported OA plasma concentrations in healthy subjects range from 0.4 to 6.0 $\mu\text{mol/l}$ (Petrarulo et al. 1998) and from <0.68 to 15.9 $\mu\text{mol/l}$ (Hönow et al. 2002). Petrarulo et al. (1998) recommend either enzymatic determination or HPLC separation followed by conductivity determination. Hönow et al. (2002) used HPLC separation coupled with an enzyme reactor. None of these methods allows discrimination between isotopically labeled and unlabeled analytes. Therefore, they were not applicable for the present study. The urinary background OA excretion reported here (Table 3) corresponds with the range of 86 to 622 $\mu\text{mol}/24\text{ h}$ published by a series of other groups (Wandzilak et al. 1991; Marangella et al. 1992; Holmes et al. 1993; von Unruh et al. 1998; Maeda-Nakai and Ichiyama 2000; Kessler et al. 2002; Siener and Hesse 2002). Possible sources for background OA include GA and endogenous glyoxylic acid (Poore et al. 1997), catabolism of ascorbic acid (Levine et al. 1996) and uptake via food (Hönow and Hesse 2002). The contribution of intermediary metabolism to background urinary OA has been estimated to be about 50% (Williams and Wandzilak 1989).

Kinetics

Derivation of kinetic parameters

The concentration–time courses of $^{13}\text{C}_2\text{-EG}$ and $^{13}\text{C}_2\text{-GA}$ in plasma of volunteers A and B can be used to predict some important kinetic parameters, using a one-compartment model (for details see for example Filser 1996). For a constant uptake rate, half the plateau concentration is reached after the first half-life (for A 2.1 h; B 2.6 h). Consequently, from Fig. 3 it becomes evident that the $^{13}\text{C}_2\text{-EG}$ plateau concentrations in blood amount to 14.4 $\mu\text{mol/l}$ (A) and 24.0 $\mu\text{mol/l}$ (B) when considering the respective “constant” inhalation rates of 358 $\mu\text{mol/h}$ (A) and 335 $\mu\text{mol/h}$ (B). The distribution volume (V_d)—related to the concentration in plasma—can be obtained using the plateau concentration (PC) and the half-life ($T_{1/2}$) since it is given by the expression $V_d = \text{inhalation rate} \times T_{1/2} / (\ln 2 \times \text{PC})$. The distribution volumes are calculated to be 75 l (A) and 52 l (B), or 0.78 l/kg bw (A) and 0.91 l/kg bw (B). These values match with those of 0.5–0.8 l/kg (reviewed in Eder et al. 1998), and are

in agreement with the assumption that the highly water soluble EG (Merck Index 1996) distributes predominantly into the aqueous phase of the body. Longer half-lives of between 3.0 and 8.6 h have been derived from cases of EG intoxication where EG concentrations in blood were in the millimolar range (reviewed in Eder et al. 1998). These differences are likely to result from saturation of EG metabolism occurring at such high concentrations.

Concerning $^{13}\text{C}_2\text{-GA}$, such kinetic calculations cannot be made, since the production rate and the distribution volume of this second $^{13}\text{C}_2\text{-EG}$ metabolite are not available from the measured data. However, a worst-case estimate for the maximum $^{13}\text{C}_2\text{-GA}$ concentration achievable as a consequence of an 8-h exposure to $^{13}\text{C}_2\text{-EG}$ can be made from the linearly increasing parts of the measured $^{13}\text{C}_2\text{-GA}$ concentration and by assuming a further continuous linear increase of the $^{13}\text{C}_2\text{-GA}$ concentration with the $^{13}\text{C}_2\text{-EG}$ -exposure time. In the present experiments, the 4-h $^{13}\text{C}_2\text{-EG}$ exposures of volunteers A and B led to maximum $^{13}\text{C}_2\text{-GA}$ plasma concentrations of 0.9 and 1.8 $\mu\text{mol/l}$, respectively (Fig. 4). For 8-h $^{13}\text{C}_2\text{-EG}$ exposures, and considering the $^{13}\text{C}_2\text{-EG}$ inhalation rates given above, a linear increase of the maximum $^{13}\text{C}_2\text{-GA}$ concentrations would lead to 1.8 (A) and 3.6 (B) $\mu\text{mol/l}$ plasma. It has to be stressed that these values are overestimates since the increase of the unknown $^{13}\text{C}_2\text{-GA}$ production rate is not constant but becomes smaller with the rising $^{13}\text{C}_2\text{-EG}$ concentration. From the observation that in both volunteers the half-lives of $^{13}\text{C}_2\text{-GA}$ were very similar (A) or identical (B) to those of the metabolic precursor $^{13}\text{C}_2\text{-EG}$, one can conclude that the elimination rate of $^{13}\text{C}_2\text{-GA}$ is determined by its production from $^{13}\text{C}_2\text{-EG}$. Consequently, an even shorter half-life of GA should be expected if GA was to be administered as such.

Kinetic extrapolations

Based on the above data, an extrapolation to workplace conditions according to the MAK-value (10 ppm, 8 h, alveolar ventilation 20 l/min per 70 kg bw; Åstrand 1983) can be carried out. Allometrically, the alveolar ventilation is calculated for volunteer A to be 24.7 l/min [$20 \times (\text{bw of A}/70)^{2/3}$], and for B to be 17.4 l/min [$20 \times (\text{bw of B}/70)^{2/3}$]. Using these values together with the individual volumes of distribution and the obtained half-lives, an exposure to 10 ppm EG ($25.7 \text{ mg/m}^3 = 0.414 \mu\text{mol/l}$) should result in maximum EG plasma concentrations of 23.0 $\mu\text{mol/l}$ (A) and 27.5 $\mu\text{mol/l}$ (B) reached on 8 h of exposure. Taking into account that the EG inhalation rate of volunteer A ($24.7 \times 60 \times 0.414 = 614 \mu\text{mol/h}$) would be 1.72 times that of the present 4-h exposure (358 $\mu\text{mol/h}$), the resulting maximum (worst-case) GA plasma concentration would amount to 3.1 $\mu\text{mol/l}$ (1.72×1.8), which has to

be added to the GA background concentration. Corresponding calculations for volunteer B yield a maximum increase over the background GA plasma concentration of 4.6 $\mu\text{mol GA/l}$. These values represent 12.0% (A) and 16.3% (B) of the corresponding background GA plasma concentrations.

Based on the relative urinary excretion data of $^{13}\text{C}_2\text{-GA}$ and $^{13}\text{C}_2\text{-OA}$ given in Table 3, corresponding increases over the urinary backgrounds resulting from EG uptake of 4.91 mmol (A) and 3.46 mmol (B)—equivalent to the 8-h exposure to 10 ppm—are predicted to be for GA and OA 12.7% and 1.7% (A), and 36.7% and 5.4% (B), respectively.

Conclusion

Workplace exposure to EG vapors under MAK conditions (10 ppm, 8 h/day, 50 W) leads to a daily EG uptake of 247 mg in a reference human of 70 kg bw. The resulting additional maximum GA and OA burdens are in the range of, or below, the unavoidable background levels. Furthermore, maximum plasma concentrations of EG and GA are rapidly decreasing after end of exposure, which becomes obvious from their short half-lives. Hereof, it has to be deduced also that both compounds cannot accumulate in daily-exposed humans (under MAK conditions). According to our data, an 8-h exposure to 10 ppm EG could lead to a maximum increase of the GA plasma concentration of less than 5 $\mu\text{mol/l}$. With the highest background GA concentration of 32.9 $\mu\text{mol/l}$, reported by Chalmers et al. (1984), this would summate to about 38 $\mu\text{mol/l}$. Considering that in rats the lowest NOEL for EG-induced nephrotoxicity was 80 mg/kg bw per day (Blood 1965), which leads to maximum GA concentrations of 144 $\mu\text{mol/l}$ blood, and that the NOEL for developmental toxicity was 500 mg/kg bw resulting in a maximum GA concentration in blood of 1723 $\mu\text{mol/l}$, we conclude that GA- and OA-induced nephrotoxic or developmental toxic effects resulting from human exposure to 10 ppm EG are highly unlikely.

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Table 1. Doses of $^{13}\text{C}_2$ -ethylene glycol inhaled by the two volunteers

Volunteer	Total dose (mmol)	Normalized dose (mg/kg body weight)
A	1.43	0.96
B	1.34	1.51

Table 2. $^{13}\text{C}_2$ -Labeled urinary ethylene glycol (EG), glycolic acid (GA) and oxalic acid (OA) expressed as a percentage of inhaled dose $^{13}\text{C}_2$ -EG

Volunteer	Amount in urine (percentage of the inhaled dose of $^{13}\text{C}_2$ -EG)		
	$^{13}\text{C}_2$ -EG	$^{13}\text{C}_2$ -GA	$^{13}\text{C}_2$ -OA
A	6.4%	0.70%	0.08%
B	9.3%	0.92%	0.28%

Table 3. Unlabeled glycolic acid (GA) and oxalic acid (OA) excreted in urine over 24 h, together with urinary excretion of $^{13}\text{C}_2$ -labeled GA and OA after inhalation of about 1.4 mmol $^{13}\text{C}_2$ -ethylene glycol, expressed as a percentage of the corresponding unlabeled acids

Volunteer	Background acid in urine		$^{13}\text{C}_2$ -Labeled acid	
	GA ($\mu\text{mol}/24\text{ h}$)	OA ($\mu\text{mol}/24\text{ h}$)	$^{13}\text{C}_2$ -GA	$^{13}\text{C}_2$ -OA
A	274	215	3.7%	0.5%
B	88	177	14.2%	2.1%